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REVIEW

Stars from the darkest night: unlocking the neurogenic potential of astrocytes in different brain regions

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ABSTRACT

In a few regions of the adult brain, specialized astrocytes act as neural stem cells capable of sustaining life-long neurogenesis. In other, typically non-neurogenic regions, some astrocytes have an intrinsic capacity to produce neurons when provoked by particular conditions but do not use this ability to replace neurons completely after injury or disease. Why do astrocytes display regional differences and why do they not use their neurogenic capacity for brain repair to a greater extent? In this Review, we discuss the neurogenic potential of astrocytes in different brain regions and ask what stimulates this potential in some regions but not in others. We discuss the transcriptional networks and environmental cues that govern cell identity, and consider how the activation of neurogenic properties in astrocytes can be understood as the de-repression of a latent neurogenic transcriptional program.

KEY WORDS: Adult neurogenesis, Neurogenic regions, Parenchymal astrocytes, Quiescent stem cells, Reprogramming, Transcriptional networks

Introduction

The adult mammalian brain contains stem cells that continuously produce new neurons (Braun and Jessberger, 2014). When these stem cells were first discovered, many hoped that they would be similar to those previously found in other organs and that they would be able to repair injuries and replace lost or damaged neurons in many parts of the brain. Since then, however, it has become clear that adult neurogenesis is important for a few, specialized functions in the healthy brain and it does not seem to be a major intrinsic mechanism for brain repair. In short, despite the presence of neural stem cells, the adult mammalian brain is not an organ that regenerates efficiently.

Adult neural stem cells are specialized astrocytes, and they are very similar to astrocytes in other parts of the brain (Götz et al., 2015). Indeed, like these stem cells, some astrocytes in the brain parenchyma even have an intrinsic ability to generate neurons. Although this ability is dormant, it can be brought out by stimulating the cells with growth factors *in vitro* (Buffo et al., 2008; Sirko et al., 2013; Shimada et al., 2012). Recently, it has been shown that some parenchymal astrocytes in the striatum can, after certain injuries, also produce neurons *in vivo* (Magnusson et al., 2014; Nato et al., 2015). The neurons generated in this way are, however, few and it is not known whether they can contribute to functional recovery in a meaningful way. Today, much research is focused on bringing out the neurogenic potential of astrocytes and on using these cells as a reservoir for new neurons in the injured brain.

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Astrocytes are located throughout the brain and are heterogeneous in form and function. Only in the subventricular zone and dentate gyrus are they clearly specialized and behave as neural stem cells. In these regions, neurogenic capacity is a product of both cell-intrinsic potential and a supportive microenvironment, but the relative importance of each is not well understood. This is even more the case for parenchymal astrocytes, whose true intrinsic potential for neurogenesis is not known. In this Review, we consider how the extent of neurogenesis varies in different regions of the adult mammalian brain. In its own way, each of these regions serves as an example of how the intrinsic neurogenic potential of astrocytes is regulated by the extracellular environment. Finally, we discuss the underlying transcriptional networks that govern cell identity and ask how environmental signals interact with these networks to allow cells to maintain latent neurogenic properties.

Adult neurogenesis in different brain regions

The subventricular zone and dentate gyrus

It might sound like a simple task to determine which brain regions are neurogenic and which are not, but depending on how 'neurogenic' is defined, different regions might qualify (Fig. 1) (Kempermann, 2011). A region may display a continuous production of new neurons; it may contain cells with an intrinsic neurogenic potential that is not realized under normal circumstances, or it may merely have an environment capable of supporting neuronal differentiation. Regardless of which definition is used, however, two regions in the mammalian brain stand out: the subventricular zone, which lines the lateral ventricles, and the dentate gyrus in the hippocampus (Fig. 1). Here, more than a thousand new neurons are generated every day (Spalding et al., 2013; Ponti et al., 2013). Newborn neurons appear hyper-excitable (Schmidt-Hieber et al., 2004), which possibly gives them a special role in information processing. In the dentate gyrus, these neurons might help the hippocampus to tell similar experiences apart and to store these experiences as separate memories (Kheirbek et al., 2012). From the subventricular zone, newborn neurons migrate to the olfactory bulb where they are involved in certain aspects of odor discrimination (Lepousez et al., 2013). In addition to its role in information processing, neurogenesis in the olfactory bulb is also important for maintaining tissue homeostasis – a role it does not have in the dentate gyrus (Imayoshi et al., 2008). Species-specific differences in adult neurogenesis do exist. Humans renew a larger proportion of dentate gyrus neurons than rodents do (Spalding et al., 2013), whereas whales and dolphins have relatively small hippocampi and apparently generate no new neurons in this region (Patzke et al., 2013). Also, in the human subventricular zone, newborn neurons do not migrate to the olfactory bulb as they do in most mammals (Sanai et al., 2011; Bergmann et al., 2012; Wang et al., 2011).

In the dentate gyrus and subventricular zone, the new neurons are produced by specialized astrocytes, which act as the brain's stem

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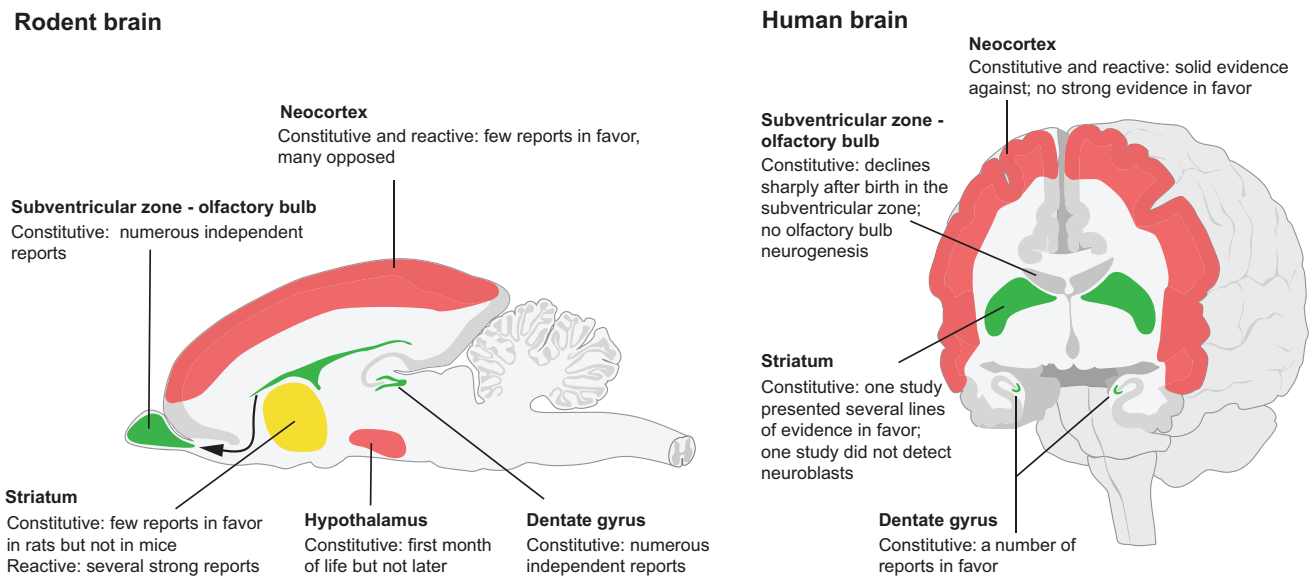

Fig. 1. The extent of neurogenesis in different regions of the adult brain of rodents and humans. In some regions, neurogenesis takes place throughout life (green), in other regions it is mostly in response to injuries (yellow) and in yet other regions, there is no strong evidence that it ever occurs in adulthood (red). Figure inspired by fig. 8-1 in Kempermann, 2011.

cells (Lim and Alvarez-Buylla, 2014). The classical definition of a stem cell calls for two central properties: multipotency, which is the ability to produce more than one cell type and self-renewal, which is the ability to retain multipotency through an indefinite number of cell divisions. Adult neural stem cells do display these properties when isolated and exposed to growth factors *in vitro* (Reynolds and Weiss, 1992; Palmer et al., 1997). But *in vivo*, these properties often emerge only on a population level. Many individual stem cells in the subventricular zone are not multipotent; they produce exclusively neurons or oligodendrocytes, but never both (Ortega et al., 2013). A stem cell might even be restricted to producing a single neuronal subtype (Merkle et al., 2007). Self-renewal, too, could be a population-level trait. In fact, individual neural stem cells in the subventricular zone may well spend most of their life in a quiescent state, becoming activated only for a few weeks of intense proliferation, followed by exhaustion of their stem cell potential (Fuentealba et al., 2015; Furutachi et al., 2015; Calzolari et al., 2015). The case might be different in the dentate gyrus. Here, some stem cells can generate both neurons and astrocytes *in vivo* (Bonaguidi et al., 2011). Indeed, inactivation of the gene *Nf1* even enables oligodendrocyte generation, which does not occur normally (Sun et al., 2015). The *in vivo* self-renewal of individual dentate gyrus stem cells is still a matter of debate. One study found that these cells rapidly exhaust their stem cell potential (Encinas et al., 2011), whereas another reported that stem cells in this region can cycle between quiescence and activation more than once (Bonaguidi et al., 2011). Even so, the recent insights from the subventricular zone suggest that neural stem cells, whose potential is brought out fully by *in vitro* conditions, might be restrained by their extracellular environment *in vivo*. This opens the possibility that in other brain regions, too, cells exist whose neurogenic potential is, in effect, hidden by a similarly restrictive environment.

Quiescent stem cells are common throughout the body, but their inactivity can make them difficult to identify as stem cells (Li and Clevers, 2010). In the subventricular zone, this difficulty has caused controversy over whether ependymal cells have stem cell properties, because these cells are able to produce neuroblasts and astrocytes only when stimulated by injuries or growth factors (Luo et al., 2015; Nomura et al., 2010; Carlén et al., 2009). As quiescence is common among the astrocyte-like neural stem cells in the neurogenic regions, it raises the tantalizing possibility that there are astrocytes in other parts of the brain that are in a similar state of quiescence.

Striatum

Until recently, the established dogma in the neurogenesis field was that the capacity for adult neurogenesis has decreased with increasing brain complexity during evolution. This dogma was challenged a few years ago when it was found that hippocampal neurogenesis takes place to the same extent in adult humans as it does in mice (Spalding et al., 2013). Even so, the finding soon afterwards that adult humans also have ongoing neurogenesis in the striatum came unexpectedly (Ernst et al., 2014). Mice, which are the most widely used laboratory animals, do not normally exhibit striatal neurogenesis under physiological conditions (Teramoto et al., 2003; Yamashita et al., 2006; Magnusson et al., 2014; Nato et al., 2015), except during a short period after birth (Inta et al., 2008). However, there is no reason to assume that the human brain should be more similar to the mouse brain than to that of other mammals. Rabbits, to which humans are equally distantly related, do exhibit adult striatal neurogenesis under physiological conditions (Luzzati et al., 2006). In fact, low levels of striatal neurogenesis have even been reported in healthy rats (Dayer et al., 2005; Arvidsson et al., 2002) and in a non-human primate species (Bédard et al., 2002), although this is controversial and other studies have not been able to replicate these findings (Benraiss et al., 2001; Wang et al., 2014).

In contrast to the dentate gyrus and olfactory bulb, where the function of newborn neurons is becoming better understood, essentially nothing is known about the purpose of adult neurogenesis in the striatum. Striatal neurogenesis in the healthy brain is, however, restricted to the medial striatum in rabbits, rats and squirrel monkeys (Luzzati et al., 2006; Dayer et al., 2005; Bédard et al., 2002). This region receives input from the limbic emotional circuitry and the auditory and visual cortex, but not primarily from motor areas, which project mostly to the lateral striatum (McGeorge and Faull, 1989). Moreover, turnover in the healthy striatum is

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largely limited to calretinin-expressing interneurons (Liu et al., 2009; Ernst et al., 2014; Luzzati et al., 2006), although the situation might be different after injury (Kokaia and Lindvall, 2012). The percentage of calretinin-positive neurons in the striatum differs markedly between species, from 1% in rodents to 10% in humans (Inta et al., 2015). It is conceivable, therefore, that increased neuronal turnover in the striatum of humans is correlated with a greater importance of this neuronal subtype.

The number of newborn striatal neurons reported in healthy humans, squirrel monkeys and rats is very low, at least compared with that in the dentate gyrus. It would be easy to conclude that neuronal turnover is therefore insignificant. However, this might not necessarily be the case. If turnover in the healthy striatum is restricted to a specific subpopulation of neurons that is very small and widely dispersed, turnover rates could be substantial as a percentage of the parental subpopulation. In the human striatum, for example, the turnover rate of the renewing cell population has been estimated at 2.7% per year (Ernst et al., 2014), which is comparable to the 1.75% estimated for the human dentate gyrus (Spalding et al., 2013). A similar case has been made for neuronal turnover in the striatum of rats (Dayer et al., 2005). In rodents, striatal neurogenesis is greatly increased after injury, particularly after stroke. However, the number of neurons that die as a result of the stroke is also very large, and one study in rats found that a mere 0.2% of dead striatal neurons had been replaced by neurogenesis 6 weeks after stroke (Arvidsson et al., 2002). At least some of these neurons are integrated into the neuronal circuitry and display functional electrophysiological properties (Hou et al., 2008), but it is not known whether so few newborn neurons can compensate for the loss of brain function that results from a stroke. Neither is it known whether humans also have increased striatal neurogenesis after stroke.

The nearby subventricular zone was long thought to be the only source of adult-born striatal neurons, based on the fact that neuroblasts can migrate into the striatum after stroke (Li et al., 2010; Zhang et al., 2009). But we and others have recently found that parenchymal astrocytes residing in the striatum can also generate neurons (Magnusson et al., 2014; Nato et al., 2015; Luzzati et al., 2014; Duan et al., 2015; Shen et al., 2015). These striatal astrocytes are triggered by certain injuries, such as stroke, to activate latent neurogenic properties and produce neurons in a burst of proliferation (Fig. 2). After stroke in rodents, thousands of newborn neuroblasts appear in the striatum over the following weeks and months (Arvidsson et al., 2002; Magnusson et al., 2014; Li et al., 2010; Zhang et al., 2009). Although many of these neuroblasts have migrated from the subventricular zone, as many as one- to two-thirds are estimated to have been generated by local astrocytes in mice (Magnusson et al., 2014). This shows that astrocytes in the striatum can act as neuronal precursor cells, at least when triggered by appropriate environmental signals. In this way, these astrocytes appear similar to adult neural stem cells – they are mostly quiescent and, when activated, proliferate in bursts to generate new neurons. Importantly, however, it is still not known whether human astrocytes also carry such latent neurogenic properties (Box 1).

Neocortex

Whether adult neurogenesis persists in brain regions other than those described above is a controversial topic. Many reports claim to demonstrate signs of ongoing neurogenesis in parts of the healthy parenchyma, such as the neocortex (reviewed in Kempermann, 2011). But most of the evidence for this is weak and often consists of ambiguous images of single cells. Therefore, it is important to use rigorous criteria when evaluating such claims.

The difficulties of trying to label brain regions as neurogenic or non-neurogenic are nowhere as obvious as in the neocortex. This is because neurogenesis requires not only a cell-intrinsic capacity to generate neurons, but it also depends on a supportive environment, which the neocortex does not have. There are cells in the neocortex, including astrocytes, with an intrinsic capacity to generate neurons. This has been demonstrated by the fact that such cells can be isolated from the injured (Buffo et al., 2008; Sirko et al., 2013; Shimada et al., 2012) or even the healthy neocortex of rodents (Palmer et al., 1999; Grande et al., 2013; Sirko et al., 2013) and coaxed into producing neurons *in vitro*. But this, on its own, does not constitute adult neurogenesis. Many groups have tried to find mature newborn neurons in the neocortex of healthy mice and primates but have so far not been successful (Magavi et al., 2000; Kornack and Rakic, 2001; Ehninger and Kempermann, 2003; Koketsu et al., 2003; Rakic, 2002). It must be mentioned that some of the astrocytes with *in vitro* neurogenic capacity isolated from the mouse neocortex actually originate in the subventricular zone, from which they migrate after injury (Faiz et al., 2015). In the healthy brain, no such migration is known to take place and in this situation, all neocortical astrocytes with *in vitro* neurogenic potential are probably locally derived (Sirko et al., 2013).

Even after injuries such as stroke, which in the rodent striatum would lead to robust production of neurons, cortical neurogenesis appears extremely limited. Although isolated newborn neurons have been reported in the rodent neocortex injured by stroke or selective neuronal loss (Ohira et al., 2010; Li et al., 2008; Jiang et al., 2001; Magavi et al., 2000; Chen et al., 2004; Brill et al., 2009), other groups have found no such neurons (Arvidsson et al., 2002; Parent et al., 2002; Diaz et al., 2013; Huttner et al., 2014). Studies that analyzed the incorporation of ^{14}C in the DNA of dividing cells observed no neurogenesis in the human neocortex, either in the healthy brain or after stroke. These results are supported by analyses of neuronal BrdU incorporation and lipofuscin content (Bhardwaj et al., 2006; Huttner et al., 2014). Importantly, the ^{14}C method has a detection level that limits the possible turnover in the human neocortex to an extremely small or short-lived subpopulation of neurons. When combined with other methods it can have an even higher sensitivity, being able to detect newborn neurons at densities as low as 1:1000 neurons (Huttner et al., 2014; Bhardwaj et al., 2006). As noted for the striatum, these results do not completely rule out neuronal turnover in the

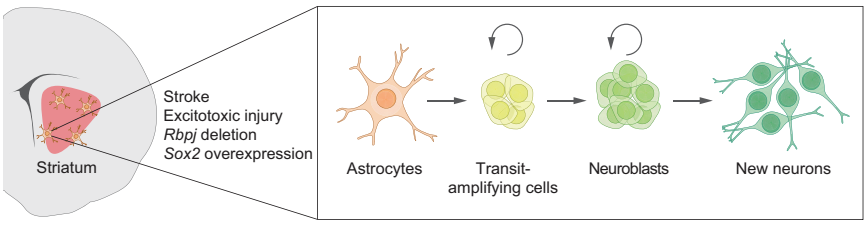

Fig. 2. Neurogenesis by astrocytes in the striatum. In the striatum (red shaded area), astrocytes can be stimulated to undergo neurogenesis after injuries like stroke, where Notch signaling is downregulated, or excitotoxic injury, by ablation of Notch-mediating transcription factor *Rbpj* or by overexpression of *Sox2*. Activated striatal astrocytes proliferate and generate transit-amplifying cells, neuroblasts and neurons.

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Box 1. Are rodent astrocytes a good model for human astrocytes?

It is not known whether rodent astrocytes are a good model for human astrocytes. The complexity of glial cells appears to increase with brain evolution (Hartline, 2011). Human astrocytes are around 17 times larger than mouse astrocytes and are more variable in form (Oberheim et al., 2009). A recent study found that human astrocytes respond to glutamate, whereas mouse astrocytes do not (Zhang et al., 2016). Human astrocytes even appear to function better: amazingly, human glial precursors have been transplanted to the mouse brain and been found to increase the cognitive capacity of the recipient mice (Han et al., 2013). Whether the increased complexity of human astrocytes comes with a difference in neurogenic capacity is not known, and the literature is ambiguous. Some studies report that cells from the human cortex can be isolated and form neurons when stimulated by growth factors *in vitro* (Palmer and Gage, 2001; Arsenijevic et al., 2001), whereas others report that they cannot (Kirschenbaum et al., 1994; Sanai and Alvarez-Buylla, 2004). The reason for this discrepancy is not known but could be related to differences in culturing protocol or post mortem time.

neocortex. There might exist a very small and widely dispersed subpopulation to which renewal is restricted. In fact, one rigorous study found evidence for a minuscule number of new interneurons in layer 6 of the rat neocortex (Dayer et al., 2005). These newborn neurons were a thousand times more sparsely dispersed compared with those in the dentate gyrus. Even if such a small neuronal population is irrelevant from a regenerative medicine perspective, its existence would be highly noteworthy because these neurons would be developing in an environment that is considered to be utterly non-neurogenic.

There are reports that describe signs of neurogenesis in other regions of the healthy adult brain than those described here. In the hypothalamus of mice, for example, neurogenesis continues for one month after birth, but not longer (Robins et al., 2013). Many other regions have been suggested to be neurogenic, but such reports have, in general, not stood the test of time (Kempermann, 2011).

Regional differences in the astrocyte phenotype

The brain regions discussed above – the subventricular zone and dentate gyrus, the striatum and the neocortex – all contain astrocytes with an intrinsic ability to produce neurons but the actual rates of neurogenesis vary dramatically between regions. In the subventricular zone and dentate gyrus, neurogenesis occurs continuously; in the striatum, mostly after certain injuries; and in the neocortex, maybe never. These differences are likely to be partly explained by cell-intrinsic differences, although the capacity of the surrounding environment to support neurogenesis could be equally important. This has been demonstrated by transplantation experiments, where cells isolated from typically neurogenic, as well as non-neurogenic regions, can generate neurons when transplanted to the neurogenic niches (Gage et al., 1995; Shihabuddin et al., 2000; Lie et al., 2002). When transplanted to the striatum, too, adult neural precursor cells have been found to generate a small amount of neurons in some studies (Zhang et al., 2003; Lim et al., 2000; Herrera et al., 1999) but not in others (Seidenfaden et al., 2006). When transplanted to the neocortex, such cells appear to generate only glia (Herrera et al., 1999). Interestingly, however, if the transplanted neural precursor cells are derived from embryos instead of adults, both the striatum and cortex appear to be more permissive to neuronal differentiation (Martínez-Cerdeño et al., 2010; Gaillard et al., 2007). Experiments like these demonstrate that the local environment has a crucial role in mediating neurogenesis, although how this is achieved and the extent to which it relies on cell-intrinsic properties of the neurogenic cells remains unclear.

Intrinsic similarities and differences between parenchymal astrocytes and adult neural stem cells

The astrocytic identity of adult neural stem cells is demonstrated by the features they share with astrocytes in the brain parenchyma. Neural stem cells express astrocyte-specific genes such as *GFAP*, *CX30* and *GLAST* (also known as *SLC1A3*) (Götz et al., 2015; Magnusson et al., 2014), they have astrocytic ultrastructure as seen in the electron microscope (Doetsch et al., 1997; Seri et al., 2001) and they also share electrophysiological features, such as low input resistance and highly negative resting membrane potential (Fukuda et al., 2003). As suggested by RNA sequencing data, metabolic characteristics, such as high glycolytic activity (Llorens-Bobadilla et al., 2015), might also be a shared feature of neural stem cells and parenchymal astrocytes (Fig. 3). As mentioned, the similarities between parenchymal astrocytes and neural stem cells even extend to an intrinsic capacity of parenchymal astrocytes to generate neurons, at least when it comes to striatal astrocytes *in vivo* (Magnusson et al., 2014; Nato et al., 2015; Duan et al., 2015; Luzzati et al., 2014) and cortical astrocytes when isolated and cultured *in vitro* (Buffo et al., 2008; Shimada et al., 2012; Sirko et al., 2013). But the relatedness between the two cell types might go even further than that. A recent study analyzed single-cell RNA-sequencing data from adult neural stem cells in the mouse subventricular zone and parenchymal astrocytes from the striatum and somatosensory cortex. This analysis showed that there might in fact not be a distinct line separating neural stem cells and parenchymal astrocytes, but rather a continuum that goes from parenchymal astrocytes, through quiescent stem cells, to activated stem cells (Llorens-Bobadilla et al., 2015). Some differences could, however, be found between the parenchymal astrocytes and the neural stem cells in this study. Genes enriched in neural stem cells compared with parenchymal astrocytes included *Cd9*, *Cd81*, *Thbs4* and *Rarres2*. Previously, prominin-1 (*CD133*) was identified as another selective marker that could be used to isolate adult neural stem cells (Beckervordersandforth et al., 2010; Walker et al., 2013). There are also morphological differences: the adult neural stem cells do not have the branched, bushy morphology of parenchymal astrocytes; in the dentate gyrus they instead display a radial projection (Seri et al., 2001) and in the subventricular zone they extend a basal process and an apical cilium to contact a blood vessel and the lateral ventricle, respectively (Mirzadeh et al., 2008).

The similarities and differences between parenchymal astrocytes and adult neural stem cells might be partly explained by their origin. During fetal development, both cell types are produced by radial glial cells, from which they retain many shared features (Götz et al., 2015). Regional differences in the radial glia lead to regional differences in the adult neural stem cells they produce (Merkle et al., 2007). Parenchymal astrocytes are also generated region by region (Tsai et al., 2012), but the extent to which this process leads to regional differences among parenchymal astrocytes is not known. For example, it is not known whether it contributes to differences in the intrinsic neurogenic capacity of striatal and cortical astrocytes.

As a matter of fact, not much is known about the general heterogeneity of parenchymal astrocytes within different brain regions. Certainly, diversity does exist: there are morphological differences between parenchymal astrocytes – for example, between those in white and gray matter (Molofsky and Deneen, 2015). Behavioral differences exist, too. For instance, live imaging of

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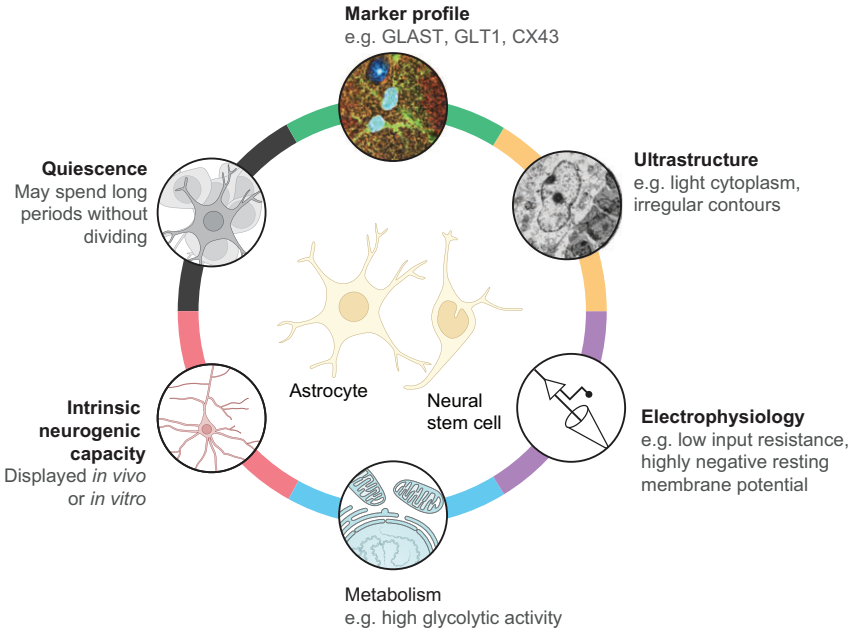

Fig. 3. Shared features of parenchymal astrocytes and adult neural stem cells. Parenchymal astrocytes and adult neural stem cells share a number of features, including (clockwise from top) marker profile (Götz et al., 2015) and ultrastructural characteristics (Doetsch et al., 1997), as well as electrophysiological (Fukuda et al., 2003) and metabolic properties, the latter being largely inferred from single-cell RNA-sequencing studies (Llorens-Bobadilla et al., 2015). Both parenchymal astrocytes and neural stem cells have an intrinsic capacity to generate neurons, although this capacity is latent in parenchymal astrocytes. Adult neural stem cells continuously produce neurons (at least when considered on the population level), but individual stem cells, like parenchymal astrocytes, might spend long time periods in quiescence (Fuentealba et al., 2015; Furutachi et al., 2015).

astrocytes after acute stab injury in the mouse somatosensory cortex revealed that different astrocytes reacted to injury in distinct ways (Bardehle et al., 2013). Despite such variety, recent studies suggest that parenchymal astrocytes are, in fact, a surprisingly homogeneous population. One single-cell RNA-sequencing analysis of cells from the somatosensory cortex and hippocampus in mice identified only two astrocyte subpopulations, whereas it detected 29 different populations of neurons (Zeisel et al., 2015). This small number of astrocyte subpopulations was confirmed in the study by Llorens-Bobadilla and colleagues, whose RNA-sequencing study even seemed to suggest that astrocytes in the cortex and striatum might be similar to one another, as they clustered closely together in a principal-component analysis (Llorens-Bobadilla et al., 2015). Intriguingly, a recent study showed that neurons control many properties of astrocytes by local release of Sonic hedgehog (Farmer et al., 2016). Studies like these suggest that many differences between astrocytes, including their neurogenic capacity, has more to do with differences in extracellular signals than with cell-intrinsic heterogeneity.

The extracellular environment of neural stem cells and parenchymal astrocytes

The transplantation studies mentioned above, where cells from both normally neurogenic and non-neurogenic regions produce neurons in some brain regions but not in others, undermine the notion of ‘stemness’ as an intrinsic and permanent cell feature. Indeed, it is well known that the very concept of stemness cannot be discussed outside the context of a cell’s environment – its niche. In the subventricular zone and dentate gyrus, the stem cell niche consists of growth factors and neurotrophins, a highly organized vasculature and extracellular matrix, ependymal cells, neurons, immune cells, astrocytes and the stem cells themselves (Ihrie and Álvarez-Buylla, 2011; Aimone et al., 2014). In parenchymal astrocytes, too, neurogenic properties seem to be dependent on environmental factors, many of which are the same as in the neurogenic niches. These include Notch signaling (Imayoshi and Kageyama, 2011; Magnusson et al., 2014), bone morphogenetic protein (BMP) signaling (Mira et al., 2010; Lim et al., 2000; Niu et al., 2013) and contact with blood vessels (Bardehle et al., 2013).

The processes by which this barrage of signals interacts with stem cells are extremely complex. Conceptually, however, the purpose of the niche is to connect the inside of a cell to its environment through receptors and to support or maintain the cell’s identity. How can cell identity be understood mechanistically? And thus, how might it be possible for an astrocyte to activate latent neurogenic properties?

Cellular and molecular mechanisms underlying the neurogenic potential of astrocytes

Cell identity is maintained by transcriptional networks

At the most fundamental level, cells can be defined by the genes they express. During cell differentiation, many transcription factors interact to build up a self-sustaining network that gives the cell its properties and locks its identity in place (Sorrells and Johnson, 2015; Holmberg and Perlmann, 2012). Such a transcriptional network is established sequentially: early sets of transcription factors activate, and are often replaced by later sets, which, in turn, activate still later sets, and so on. In mouse olfactory neuron progenitors, for example, *Ascl1* expression is followed by *Ngn1* expression, which is then followed by expression of *Neurod1* (Ernsberger, 2015). As differentiation proceeds, transcription factors that specify competing lineages are repressed (Imayoshi and Kageyama, 2014; Graf and Enver, 2009). The end product, a differentiated cell, is constructed by a final transcriptional network that is stabilized by self-regulating interactions of its component factors (Hobert, 2008). In this way, the transcriptional networks that define cell identity might be seen as stable states – each acting as an attractor towards which the less-stable transcriptional networks within immature cells will gravitate (Graf and Enver, 2009; Holmberg and Perlmann, 2012).

Transcription factors that are important during differentiation often remain expressed in the mature cell (Hobert, 2008). A cell can be vulnerable to perturbations of such important factors and even single gene deletions can cause it to switch into a different but related cell type. For instance, ablation of *Prox1* in lymphatic endothelial cells leads to transdifferentiation into blood endothelial cells (Johnson et al., 2008). Similarly, ovary cells are reprogrammed to testis cells, and vice versa, by the deletion of *Foxl2* or *Dmrt1*, respectively (Uhlenhaut et al., 2009; Matson et al., 2011).

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Surprisingly, however, there are some cases where deletion of a gene that is crucial for the development of a particular cell type has little effect in the differentiated cell. For example, even though knockout of the transcription factors *Pet1* or *Lmx1b* in serotonergic neurons during early differentiation causes a loss of this cell type (Cheng et al., 2003; Ding et al., 2003), ablation of either factor in fully differentiated serotonergic neurons causes only mild abnormalities (Song et al., 2011; Liu et al., 2010). One explanation for this phenomenon might be that redundant factors act to stabilize transcriptional networks during differentiation. Such a mechanism would benefit cells where phenotypic stability is required for an entire lifetime, such as neurons. In these cells, redundancies could act like buttresses that make the transcriptional network resistant to perturbations.

Robust phenotypic stability is essential for some cells, but in other cell types, maintaining a certain degree of plasticity might be more important. In such cases, it might be that unstable transcriptional networks represent a 'deliberate' mechanism of plasticity (Holmberg and Perlmann, 2012). In such cells, perturbations of a single key gene or signaling pathway might be enough to throw their transcriptional network into flux and force it toward the closest attractor state. Mechanistically, this would be similar to reprogramming to induced pluripotent stem cells, where an initially stochastic phase is followed by a highly orchestrated establishment of cell fate (Buganim et al., 2013). It has been proposed that cellular plasticity should be viewed as a metastable state, in which cells co-express two or more mutually repressive transcriptional networks (Graf and Enver, 2009). Such cells would be balanced on the edge between two competing networks, ready to execute an identity switch if triggered by small perturbations. In the brain, one cell type that could potentially be set up in such a way are astrocytes, because it has already been shown that injuries can trigger at least some astrocytes to turn on a neurogenic program (Magnusson et al., 2014).

Similar cell types share similar transcriptional networks (Neph et al., 2012). Astrocytes and adult neural stem cells share many attributes, which might be why small perturbations are enough to nudge some astrocytes into behaving like neural stem cells. The question is: how do striatal astrocytes orchestrate this identity switch and could astrocytes in other regions also be instructed to do this? To approach this question, we look at studies where glial cells have been reprogrammed *in vivo*. These experiments suggest that glia can be forced to produce neurons in two fundamentally different ways: through direct transdifferentiation or via a transient progenitor state.

Making neurons: direct transdifferentiation versus activation of a progenitor state

A number of studies show that overexpression of key transcription factors can force astrocytes and NG2 glia to transdifferentiate into neurons *in vivo* (Guo et al., 2014; Heinrich et al., 2010, 2014; Liu et al., 2015; Su et al., 2014). The reprogramming factors used are often chosen for their important roles in neuronal differentiation during brain development. For instance, Ascl1 is a pioneer factor that makes chromatin more accessible (Raposo et al., 2015), allowing additional transcription factors to access relevant promoters (Wapinski et al., 2013). Such additional factors, such as Brn2, Myt1l, Zfp238, Ngn2, NeuroD1, Pax6 and Dlx2, are expressed during normal neuronal differentiation and specify a broad neuronal identity (Wapinski et al., 2013; Guo et al., 2014; Pang et al., 2011; Heins et al., 2002; Grande et al., 2013; Heinrich et al., 2010). They work by superimposing a neuronal transcriptional network on to that of the starting cell, leading to transdifferentiation within a few days. Although direct transdifferentiation is a powerful approach, it occurs through mechanisms that are different to those during normal differentiation (Péron and Berninger, 2015; Holmberg and Perlmann, 2012). It is also different to the way astrocytes produce neurons after stroke, which happens via proliferative intermediate cells (Magnusson et al., 2014). Transdifferentiation does not require cell division (Di Tullio and Graf, 2012), which means that each generated neuron consumes one starting cell. Clearly then, converting glia into neurons will disrupt the ratio of glia to neurons, which is roughly 4:1 in the human cortex (Lent et al., 2012). It is not known what the functional effect of this would be, but it is likely that an appropriate ratio of glia to neurons is required for proper neural circuit function and an aberrant ratio might be implicated in many neurodevelopmental disorders (Sloan and Barres, 2014). An attractive alternative then, is to generate many neurons from a single starting cell, which would involve the creation of a transient, proliferative progenitor cell type.

Instead of direct transdifferentiation, some single-gene manipulations can cause cells to dedifferentiate to a multipotent intermediate state. In the hematopoietic lineage, deletion of *Pax5* in committed pro-B cells, or *GATA2* in mast cells, activates a multipotent phenotype (Mikkola et al., 2002; Ohmori et al., 2015). In the mouse brain, deletion of the Notch mediator *Rbpj* or overexpression of *Sox2* activates a proliferative neurogenic state in astrocytes in the striatum, and in the case of deletion of *Rbpj*, also in a narrow band along the medial cortex (Fig. 2) (Magnusson et al., 2014; Niu et al., 2013). This artificially induced neurogenesis appears to be indistinguishable from how striatal astrocytes generate neurons after stroke – a process that is indeed triggered by downregulation of Notch (Magnusson et al., 2014). When activated, a neurogenic astrocyte undergoes a proliferative burst to produce 30-40 neuroblasts (Magnusson et al., 2014). This means that astrocytes could, in theory, produce many neurons without being completely depleted in the process, at least within the striatum. But what would it take to activate a similar neurogenic program in astrocytes outside the striatum and medial cortex? To approach an answer to this question, we will look to how this process occurs in striatal astrocytes in order to understand how the latent neurogenic program of astrocytes is regulated.

Striatal neurogenesis might occur through the activation of a repressed transcriptional program

The stages through which deletion of *Rbpj* and overexpression of *Sox2* stimulate the generation of neurons from striatal astrocytes appear identical. In both cases, astrocytes start proliferating after 2-3 weeks and generate clusters of Ascl1-positive transit-amplifying cells and DCX-positive neuroblasts. It might seem strange that two such different manipulations should trigger these indistinguishable results. However, if one considers that quiescent stem cells are defined by two competing transcriptional networks, it makes sense. Within this paradigm, a cell-identity switch can happen either if the current transcriptional network is destabilized or if the competing network is stabilized. *Rbpj* deletion and *Sox2* overexpression might illustrate these very two situations (Fig. 4). Notch signaling promotes astrocyte differentiation, partly through suppression of Ascl1, which regulates a large proportion of neurogenic genes (Llorens-Bobadilla et al., 2015; Andersen et al., 2014; Kanski et al., 2014). Notch then remains active in differentiated astrocytes (Magnusson et al., 2014). Given the central role of Notch in regulating cell fate decisions in many different contexts (Artavanis-Tsakonas et al., 1999), it is tempting to postulate that, in striatal astrocytes, it could be acting as a node

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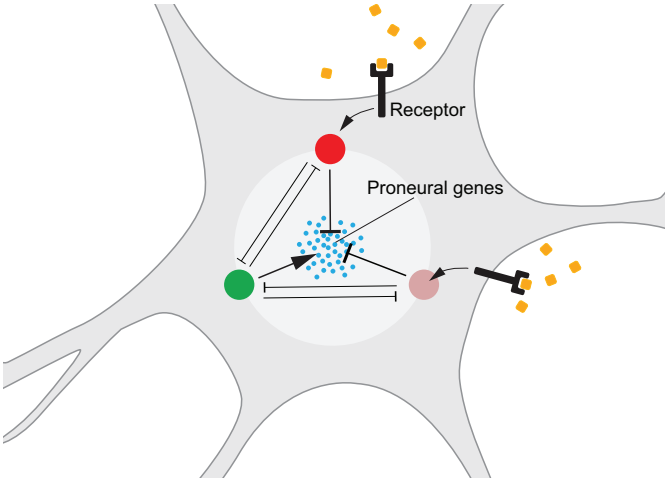

Fig. 4. A conceptual model of the mechanisms regulating neurogenesis in parenchymal astrocytes. In a hypothetical and simplified model, proneural genes (blue) in astrocytes are regulated by activators (green) and repressors (red), which in addition repress each other. Depending on which regulators are stronger, proneural genes are either suppressed or expressed. The regulators can themselves be regulated by signals from the extracellular environment (orange). In striatal astrocytes, decreased Notch signaling or *Sox2* overexpression can activate proneural genes, but in non-striatal astrocytes, proneural genes might also be additionally repressed by redundant, stabilizing regulators (pink) that prevent activation of neurogenesis.

around which two competing transcriptional networks can pivot. If this were true, then *Rbpj* deletion or stroke-induced downregulation of Notch might destabilize the astrocytic network so much that the competing neurogenic network gains the upper hand. As the Notch-mediated repression of Ascl1 is lifted, Ascl1 reaches levels where it is capable of triggering a self-sustaining feed-forward induction of the neurogenic network. Newly expressed neurogenic factors, in turn, engage in cross-repression of the astrocytic network, and the net effect of these two mechanisms is enough to cause a stable cell-identity switch from astrocyte to neural progenitor. Whereas deletion of *Rbpj* might promote neurogenesis by destabilizing the astrocytic network, Sox2 could work by stabilizing the neurogenic network. This stem cell-associated transcription factor primes neurogenic genes for activation (Amador-Arjona et al., 2015). In striatal astrocytes, Sox2 could be a crucial component of the latent neurogenic transcriptional network and, although it is expressed in parenchymal astrocytes, Sox2 acts in a dose-dependent manner in neural stem cells (Hagey and Muhr, 2014). Overexpression could be what it takes to stabilize the neurogenic network enough to repress the astrocytic network and initiate neurogenesis.

From this perspective, the activation of a latent neurogenic program can be understood as the sudden switch to a repressed transcriptional network. This paradigm presents several predictions. First, although *Rbpj* and *Sox2* have been shown to serve as important 'switches' (Magnusson et al., 2014; Niu et al., 2013), any gene that is important for stabilizing an astrocytic or neurogenic transcriptional network could be manipulated to achieve the same result. Candidates for such genes could be those that are important for instructing the astrocyte phenotype, such as components of the BMP, Sonic hedgehog and peroxisome proliferator-activated receptor (PPAR) signaling pathways (Cahoy et al., 2008; Michelucci et al., 2015; Gallo and Deneen, 2014). Second, although irreversible genetic manipulations have been used to activate a latent neurogenic program (Magnusson et al., 2014; Niu et al., 2013), it should be possible to achieve a stable cell-identity switch using only transient interventions; for example, treatment with pharmaceutical compounds. Once an identity switch has occurred, feed-forward and cross-repressive mechanisms should operate to make the new network stable, provided that the environment is permissive. This has been conceptually demonstrated in the lung, where transient Notch manipulation leads to permanent transdifferentiation of club cells to ciliated cells (Lafkas et al., 2015). A similar process is likely to occur when Notch signaling in astrocytes is reduced in response to stroke (Magnusson et al., 2014). Third, astrocytes that are governed by a highly redundant transcriptional network would be more resistant to phenotypic change than those with relatively low redundancy. If this is true, it might explain why deletion of *Rbpj* did not activate a neurogenic program in astrocytes outside the striatum and medial cortex (Magnusson et al., 2014). In other words, the lack of neurogenesis in other regions is not necessarily because astrocytes outside these regions lack an intrinsic neurogenic capacity (Buffo et al., 2008; Sirko et al., 2013) but rather because their identity might be supported by redundant signaling pathways, which would require more than a single perturbation to disrupt. Identifying and targeting additional pathways for manipulation may break through this network redundancy and trigger neurogenesis from these astrocytes.

A question of quiescence

Quiescence is a reversible cell cycle arrest where cells are poised to rapidly re-enter the cell cycle in response to environmental cues. For example, satellite cells in muscle, and astrocytes in the striatum, react to certain injuries by initiating division within a few days (Ogawa et al., 2015; Magnusson et al., 2014). The genes and pathways that regulate the quiescent state are different in different tissues. This is probably because evolution uses whatever signaling pathways happen to be at hand and rewires them into new functional circuits (Kashtan and Alon, 2005; Milo et al., 2002). For instance, Notch signaling promotes quiescence in muscle satellite cells and striatal astrocytes (Dumont et al., 2015; Magnusson et al., 2014), but is not required for hematopoietic stem cell maintenance (Maillard et al., 2008), and even promotes exit from quiescence in interfollicular epidermis and hair follicles (Estrach et al., 2008; Williams et al., 2011). Even so, it appears that quiescent stem cells throughout the body share some common features, including low rates of metabolism, low levels of protein synthesis and expression of negative regulators of cell division, although for neural stem cells, the low metabolic state is largely inferred from RNA-sequencing data (Cheung and Rando, 2013; Llorens-Bobadilla et al., 2015; Shin et al., 2015; Furutachi et al., 2015).

Activation from quiescence

Quiescent stem cells must respond quickly to injuries and therefore monitor environmental signals closely. Muscle stem cells, for instance, appear 'highly strung' in that even distant injuries prompt them to enter into an alert state in which they are poised for activation (Rodgers et al., 2014). This is useful, because the first cell division after activation takes a particularly long time to complete (Siegel et al., 2011). In parenchymal astrocytes, too, injury signals regulate the exit from quiescence (Bardehle et al., 2013). And in addition, these signals modulate the intrinsic neurogenic capacity of these cells (Sirko et al., 2015; Michelucci et al., 2015). In striatal astrocytes, the latent neurogenic program is governed by Notch signaling (Magnusson et al., 2014). In addition to Notch, however,

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other injury signals appear to be acting synergistically to bring out this neurogenic capacity. After deletion of *Rbpj* in the uninjured mouse brain, most astrocyte-derived neuroblasts appeared in the medial striatum (Magnusson et al., 2014). But a small injury caused by a needle insertion was enough to elicit the response by *Rbpj*-deficient astrocytes in the lateral striatum. It is very interesting to speculate that some striatal astrocytes had been 'primed' for neurogenesis by Notch inactivation, but that an additional push, such as the exit from quiescence promoted by injury signals, was necessary to initiate neurogenesis. Intriguingly, however, reactive astrogliosis, an astrocyte response to injury, is not a prerequisite for activation of the neurogenic program. In the uninjured striatum, *Rbpj*-deficient astrocytes entered the neurogenic program without any signs of reactive astrogliosis, such as GFAP expression or hypertrophy (Magnusson et al., 2014). Even after stroke, some Ascl1-positive astrocytes lacked signs of reactive astrogliosis, whereas others were reactive. Conversely, of all reactive astrocytes after stroke, only a minority expressed Ascl1. This shows that the transcriptional programs that govern reactive astrogliosis and neurogenesis in striatal astrocytes are not necessarily coupled, although they seem to be mutually compatible. It could therefore, in theory, be possible to modulate these two programs separately in order to strike the right balance between astrocyte-mediated scarring and neurogenesis.

The mechanisms by which environmental signals regulate activation from quiescence are likely to be complex. A multitude of environmental inputs feed into intracellular signaling circuits that compute an output – in this case whether or not to remain quiescent. Reductionist experimental approaches with single-gene deletion or overexpression do not recreate this fine-tuned complexity, but are beginning to carve out some of the major signaling pathways involved. Notch and BMP are two important pathways in neural stem cells (Mira et al., 2010; Imayoshi and Kageyama, 2011; Magnusson et al., 2014; Niu et al., 2013; Lim et al., 2000). Another important player is Sonic hedgehog, which is present in the cerebrospinal fluid after invasive injury. This signaling molecule also activates the neurogenic properties of astrocytes *in vitro* (Sirko et al., 2013), perhaps by interacting with the Notch pathway (Kong et al., 2015). Galectins are another example, because they have been shown to be important for proliferation of reactive astrocytes (Sirko et al., 2015). The single-cell RNA-sequencing study by Llorens-Bobadilla et al. identified, among others, *Sox9*, *Id2* and *Id3*, as genes characteristic of the quiescent state in neural stem cells, whereas the activated state was found to be associated with the expression of *Egr1*, *Fos*, *Sox4*, *Sox11* and *Ascl1* (Llorens-Bobadilla et al., 2015).

After initiation of neurogenesis, the later stages of neuronal maturation also require permissive environmental signals. Indeed, ectopic neuroblasts often die or mature into glia in the brain parenchyma (Arvidsson et al., 2002; Li et al., 2010). Furthermore, despite the appearance of thousands of striatal neuroblasts after stroke, only a small percentage of them survive as neurons in rodents (Magnusson et al., 2014; Arvidsson et al., 2002). In the dentate gyrus and olfactory bulb, that number is around 50% (Dayer et al., 2003; Petreanu and Alvarez-Buylla, 2002). Even worse is the maturation capacity in the medial cortex, where neuroblasts generated by *Rbpj*-deficient astrocytes do not develop even beyond a tightly packed cluster stage (Magnusson et al., 2014). Neuronal differentiation can, however, be improved by supplying signals that promote neuronal maturation (e.g. BDNF or histone deacetylase inhibitors) or inhibit glial differentiation (e.g. the BMP antagonist Noggin) (Niu et al., 2013; Lim et al., 2000). Overcoming the anti-neurogenic environment is one of the biggest hurdles on the way towards regenerative therapies in the brain.

It is not known whether striatal astrocytes can re-enter quiescence after having activated their neurogenic program, or if they are irreversibly consumed by this process. As mentioned previously, it is not clear whether all adult neural stem cells exhaust their stem cell potential after a few rounds of division (Encinas et al., 2011; Calzolari et al., 2015), or whether some might be able to go back to a quiescent state (Bonaguidi et al., 2011). In muscle, environmental signals can make satellite cells re-enter quiescence after being activated. This re-entry process depends on a reactivation of the Notch signaling pathway (Kuang et al., 2007). Because the latent neurogenic potential of striatal astrocytes is triggered by a downregulation of Notch signaling, it is interesting to speculate whether reactivation of Notch could return these astrocytes to the quiescent state, from which they might be capable of being reactivated over and over again.

Conclusions

All cells in the body share the same genetic information; therefore, theoretically, every cell has the potential to change phenotype and function at any point in time. And yet, for the most part, they do not. Most cells secure their differentiated state with transcriptional networks stabilized by redundancies and epigenetic modifications. Even so, some differentiated cell types are particularly amenable to cell cycle re-entry and activation of progenitor properties. These quiescent stem cells have latent transcriptional networks that are poised for activation, possibly held in check by 'nodes', which are proteins vulnerable to fluctuations in environmental signals. Relatively labile cell identities, such as astrocytes, have now begun to be identified.

Neural stem cells in the subventricular zone and dentate gyrus are specialized astrocytes that are quite clearly supported by a transcriptional network that facilitates exit from quiescence and subsequent neurogenesis. In the striatum, the transcriptional network that underlies neurogenesis is latent and becomes activated only after certain injuries. In most of the neocortex and elsewhere, the intrinsic neurogenic potential of astrocytes remains hidden and is seen only when cells are stimulated *in vitro* or genetically manipulated *in vivo*. Thus, having the right transcriptional network to enable neurogenesis is only part of the mechanism underlying neurogenesis. As discussed in this Review, the environment in which cells are located and the signals with which they are presented following injury feed directly into this network to either permit or block neurogenesis. Understanding what these signals are, how they vary according to brain region and how they integrate into the transcriptional network in order to control neurogenesis will be an interesting challenge for the future.

Classical experimental approaches where one or a few genes are overexpressed or deleted are very valuable for understanding the role of individual genes in complex cellular processes such as differentiation and stem cell maintenance. Recent work has identified switching mechanisms that activate a latent neurogenic program in striatal astrocytes (Magnusson et al., 2014; Niu et al., 2013). Redundancies in the transcriptional network might be contributing to increased phenotypic stability in astrocytes in other regions. If it were possible to uncover these redundancies, then it might be possible to destabilize the differentiated state and activate a latent neurogenic program in many parts of the brain. Such an advance would mark an important step toward the use of endogenous glial cells as a reservoir for neurogenesis in the injured brain. Today, with approaches such as single-cell RNA

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sequencing, we are beginning to learn how whole transcriptomes are regulated in the context of complex cellular behaviors. However, the signaling networks that occur in nature have been generated by natural selection – a process hypothesized to have generated networks with many non-functional interactions and redundancies (Sorrells and Johnson, 2015). Such functional and non-functional complexity would make it difficult to extract the relevant information from transcriptome data. We believe that although these experimental approaches will continue to provide much valuable information, important conceptual insights could also be made by attempting to construct, from the bottom up, minimal synthetic signaling networks capable of performing the same computational tasks as those observed in nature (Bashor et al., 2010). If we can construct the networks that govern complex behaviors such as cell differentiation, surely we can then be said to understand them, and in turn, begin to manipulate them towards better outcomes for neuronal repair.

Competing interests

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